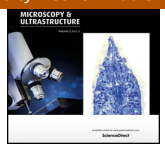
Journal of Microscopy and Ultrastructure 2 (2014) 127–136

Journal of Microscopy and Ultrastructure

journal homepage: www.elsevier.com/locate/jmau



Original Article

Utility of immunohistochemical markers in differential diagnosis of follicular cell-derived thyroid lesions

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ARTICLE INFO

Article history:
Received 14 December 2013
Received in revised form 8 February 2014
Accepted 18 February 2014
Available online 7 March 2014

Keywords:
Thyroid carcinoma
CD56
HBME-1
Gatectin-3
CK19

ABSTRACT

Background: Differentiating the different follicular derived lesions from each other can be challenging. Although immunohistochemistry is generally accepted as a useful ancillary technique in the diagnosis, controversy exists regarding the best marker or combination of markers to distinguish each lesion from its mimics. In this study, we aimed at evaluating multiple markers to compare their sensitivity and usefulness, and to find out if a combination of the evaluated markers can be of additional value in discriminating thyroid lesions.

Methods: The study included two groups of follicular derived thyroid lesions. Immunohistochemical evaluation of CD56, HBME-1, Gaectin-3 and CK19 was done for the two groups. The sensitivity and the specificity for each marker and their combination in the diagnosis were calculated.

Results: Each studied marker was sensitive and specific for certain thyroid lesion but the sensitivity and the specificity were increased when two or more markers from the panel were used together.

Conclusions: Although no single immunohistochemical marker by itself is completely sensitive and specific for follicular thyroid lesions, the combination of CD56, HBME-1, Gaectin-3 and CK19 attains high sensitivity and specificity in differentiating follicular derived thyroid lesions.

1. Introduction

Follicular derived thyroid disease refers to the presence of a benign or malignant solid nodule, a multinodular gland, Grave's disease, or thyroiditis. The microscopic distinction by conventional histology between benign and malignant lesions may be difficult [1,2]. Most of the discovered nodules are benign. More than 80% of the malignancies present in palpable thyroid nodules are papillary thyroid carcinoma (PTC) followed by follicular carcinoma (FC) [3–5].

The "gold standard" in diagnosis of thyroid nodules is pathologic evaluation using routine hematoxylin and eosin (H&E) staining. However, morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPC) is common which is characterized by an almost exclusive follicular growth pattern and a set of nuclear features identical to those of the classic type of PTC [6,7]. Diagnostic dilemma may arise when an encapsulated nodule with a follicular pattern of growth exhibits clear nuclei with grooves and so distinguishing

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http://dx.doi.org/10.1016/j.jmau.2014.02.004
2213-879X/

follicular adenoma (FA) from encapsulated FVPTC becomes difficult. There are several other thyroid lesions that may contain papillary processes with nuclear features, which pose diagnostic difficulties with PTC [8]. Multinodular goiter (MNG) with delicate papillary budding and focal nuclear clearing may be confused with PTC [9,10]. Also a new category emerged that was named Follicular Neoplasm/atypical cells of undetermined significance (AUS). This category accounts for 10–25% of all cases and represents a therapeutic problem because of the low risk of malignancy [2].

A growing number of some promising immunohistochemical (IHC) markers for the differential diagnosis of thyroid lesions have emerged, including CD56, Hector Battifora mesothelial (HBME-1), galectin-3 (Gal-3) and CK19 but till now none of them are conclusive [5,7].

CD56 is a neural cell adhesion molecule. Its expression may affect the migratory capability of tumor cells. Hence it is not surprising that loss of CD56 correlates with metastatic potentials and poor prognostic outcome in some malignancies [7]. It has been reported to be expressed in normal thyroid follicular cells with frequent low expression in malignant thyroid tumors especially PTC [10].

HBME-1 is an antigen on the surface of mesothelial cells. In thyroid neoplasms, Husain et al. [11] study showed that HBME-1 was positive in PTC and FC. However, none of them have shown a diagnostic accuracy sufficient for using a single antibody in the diagnosis of malignant thyroid neoplasms. Besides, no studies have been performed to determine whether HBME-1 is a useful diagnostic tool for distinguishing FA or Follicular Neoplasm/atypical cells of undetermined significance (AUS) [12].

Galectin-3 is a component of the β-galactoside binding lectins whose function is still unclear. It appears to be involved in the cell–cell and cell–matrix modulation. Therefore, it could play a role in the malignant transformation of thyroid cells and it is expressed in a high proportion of carcinomas, especially of the papillary type [11]. Recently, galectin-3 is initially shown to have utility in the differential diagnosis between benign and malignant thyroid lesions [2]. But some recent studies suggest that it is not reliable [4,9,12].

Cytokeratin 19 (CK19) is a type I intermediate filament protein and is widely present in simple epithelial cells [7]. Several studies demonstrated strong and diffuse positivity in malignant thyroid tumors; however, it is not specific to malignancy [3,11]. Several studies have shown conflicting results regarding the usefulness of CK19 as a diagnostic marker in thyroid lesions [7,13].

Most studies have evaluated the single expression of markers in various thyroid lesions and a few reports have studied the combined expression of markers [14,15]. Therefore, in this study, we evaluated the usefulness of using a panel of four markers (CD56, Gal-3, HBME-1, and CK-19) individually and in combination and their diagnostic value, in various follicular derived thyroid lesions. Our aim was to identify the diagnostic role of these markers in the follicular morphological mimics to determine their sensitivity and specificity in differential diagnosis of thyroid nodules.

Table 1
The distribution of the studied cases.

Studied cases		
Normal ($n=10$)		
Lesions ($n=70$)	First group ($n=20$)	Grave's disease ($n=4$)
		MNG ($n=5$)
		Hashimoto's thyroiditis ($n=4$)
		FA ($n=7$)
	Second group ($n=50$)	PTC ($n=22$)
		FTC ($n=15$)
		WDTs-UMP ($n=7$)
		FT-UMP ($n=6$)

2. Materials and methods

2.1. Tissue specimens

Thyroid gland lesions from January 2009 to January 2013 were searched through the database charts at the pathology department of Tanta University Hospital. Demographic information, gender, type of surgery, clinical data, tumor stage, treatment, tumor recurrence and follow up were reviewed. The study included 25 male and 45 female patients with a median age of 32.5 years (range 13–78 years). The material of this retrospective study included 70 specimens of surgically removed, formalin-fixed and paraffin embedded thyroid lesions. Furthermore, another 10 samples of randomly chosen normal thyroid tissue obtained from radical laryngectomies for laryngeal carcinomas were included. This study was approved by the ethical committee of the hospital. The tissue processing and the general histological report were performed as described previously by Ozolins et al. [16]. The diagnosis and typing of thyroid pathology were performed according to the World Health Organization Classification [17].

For simplicity and practical clinical considerations, the 70 selected thyroid lesions were divided into two groups: benign (including nonneoplastic and neoplastic) and malignant. The first benign group (20 cases) included 4 Grave's disease cases; 5 MNG cases; 4 Hashimoto's cases and 7 FA cases, while the malignant group (50 cases) had inclusion criteria as follows: differentiated thyroid cancer originating from follicular epithelial cells except a Hürthle cell variant. This included 22 PTC cases; 15 FTC cases; 7 cases of well differentiated tumors of unknown malignant potential (WDTs-UMP) and 6 cases of follicular tumor of unknown malignant potential (FT-UMP). The 22 PTC cases were further classified into 14 cases of classic PTC and 8 cases of FVPCs (Table 1).

For the diagnosis of FA, they were defined as completely encapsulated follicular tumors with homogeneous architecture and morphology, without capsular and vascular invasion [7]. While for PTC we followed the histological criteria proposed by Chan [18], which are divided into major and minor features. The major features include: (1) nuclei are ovoid; (2) nuclei are crowded; (3) nuclei show a clear chromatin; and (4) psammoma bodies are found. If one of the four features was lacking, four or more of the following features may occur: (1) presence of abortive papillae; (2) irregular shaped follicles; (3) dark colloid; (4)

presence of nuclear pseudoinclusions; or (5) multinucleated histiocytes in follicle lumen. Tumors were classified as FVPC if they were composed completely or almost entirely (99% of the tumor) of follicles lined by cells that had the nuclear features of PTC [8]. FC was diagnosed based on the presence of follicular proliferation with complete capsule and full capsular penetration and/or vascular invasion [18]. WDT-UMP was represented by an encapsulated tumor composed of follicular cells having incompletely developed papillary carcinoma-type nuclear changes. In these tumors, there was no vessel invasion, while capsular penetration was either absent or questionable. While FT-UMP was defined as an encapsulated tumor with follicular architecture, having incomplete or questionable capsular penetration, but neither vascular invasion nor papillary carcinoma-type nuclear changes [19].

2.2. Immunohistochemistry

All 80 samples (70 thyroid lesions and 10 normal thyroid tissues) were subjected to immunohistochemical staining with CD56, HBME-1, Gal-3 and CK-19 antibodies. The sections were deparaffinized in xylene and rehydrated through absolute alcohol. Antigen retrieval in citrate buffer (pH9 Lab vision cat#AP9003) was used after the sections were treated in a microwave at 8 W for 5–6 min, then at 3 W for 10 min, and the sections were then left to cool for 20 min. Peroxidase and protein blocks were done. After that the slides were incubated overnight with the primary antibodies at room temperature using CD56 antibody (clone 123C3; 1:100; Dako, Glostrup, Denmark); anti-HBME-1(clone HBME-1; 1:50; Dako, Glostrup, Denmark); anti-Gal-3 monoclonal antibody NCL-Gal3, dilution 1:200 (Novocastra, Newcastle, UK) and anti-CK-19 polyclonal antibody, dilution 1:100 (DakoCytomation) followed by rinsing in PBS (phosphate buffered saline, pH 7.6). This was followed by the secondary biotin conjugated antibody for 1 h and finally the peroxidase conjugated streptavidin for another hour. Diaminbenzidine tetrachloride (DAB) was added for 25 min, and then counterstained in Hematoxylin, followed by dehydration, clearing and mounting. The slides of positive and negative control were included in each run. Positive control for CD56 was neuroblastoma, mesothelioma cells for HBME-1, histiocytes for galectin-3 and skin for CK19. Negative controls were done by excluding primary antibody and its replacement with PBS [20].

2.3. Interpretation of immunohistochemical staining of the studied markers

According to Park et al. [20], strong and complete membranous expression with or without cytoplasmic staining of the cells qualified the case as positive for CD56.

We regarded cells as immunoreactive for HBME-1 when the signal was clearly observed in the cytoplasms and/or the membrane according to Yasuhiro et al. [4].

The cells were regarded as positive for Gal-3 when immunoreactivity was clearly observed in their nucleus and/or cytoplasm [9].

A positive membranous expression with or without cytoplasmic staining in 10% or more of neoplastic cells qualified the case as “positive (+)” for CK19 [11].

2.4. Scoring for the immunomarkers

A semiquantitative assessment of immunohistochemical scoring was performed. For all antibodies, immunoreactivity was considered positive if >10% of follicular epithelial cells stained [21]. The immunoreactivity was scored as negative, focally positive (+: less than 25%), positive (++: 25–50%) or diffusely positive (+++: more than 50%), based on the extent of the reaction [4,6,20].

2.5. Statistical analysis

Data were analyzed using the SPSS program Version 15. Comparison of qualitative variables between groups was done using the Chi-square or Fisher’s exact test. The sensitivity and the specificity for each marker and their combination in the diagnosis were calculated. Probability values less than 0.05 were considered significant [22].

3. Results

3.1. Immunohistochemical expressions in normal thyroid tissue

Strong CD56 positive expression was found in the 10 samples of normal thyroid tissue 10/10 (100%). HBME-1 immunoreactivity was not observed in normal follicular epithelium, but was observed in scattered histiocytes. Gal-3 expression was completely negative in the normal thyroid tissue. Strong CK19 expression was observed in the 10 normal thyroid samples (100%).

3.2. Immunohistochemical expression in the studied thyroid lesions (Table 2)

3.2.1. 1-CD56 expression in the studies lesions

Among the first group, positive CD56 expression was observed in 16/20 cases (80%), which included 3/4 cases of Grave’s disease (75%), 4/5 cases of MNG (80%), 3/4 cases of Hashimoto’s thyroiditis (75%) and 6/7 cases of FA (86%) [Fig. 1a]. All of the positive cases displayed strong CD56 expression (score +++). No statistical significant difference was found among this group as regards CD56 expression ($P = 0.9$).

Among the second group, positive CD56 expression was observed in only 5/50 cases (10%), which included 2/14 classic PTC (14%, score ++). Fig. 2b shows example of the negative PTC cases]. Besides, 1/8 cases of FVPC (13%, score +) [Fig. 1c], 2/15 cases of FC (13%, score +) [Fig. 1d], no positive WDT-UMP nor FTs-UMP (0%). No statistical significant difference was found among this group as regards CD56 expression ($P = 0.5$) but CD56 distinguished the second group from the first group with a high statistically significant difference ($P = 0.001$).

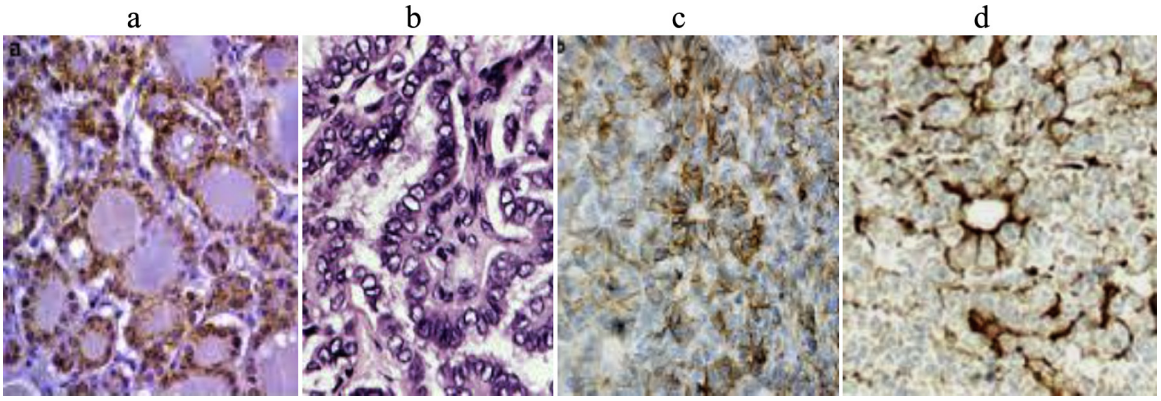

Fig. 1. Immunohistochemical staining of CD56 in FA showing (+++) (a), PTC (−) (b), FVPC (+) (c) and FC (+) (d) [streptavidin biotin 200×].

3.2.2. 2-HBME-1 expression in the studied lesions

HBME-1 signal was detected predominantly in the cytoplasm.

Among the first group, positive HBME-1 expression was observed in only 4/20 cases (20%), which included 1/4 cases of Grave's disease (25%), 1/5 cases of MNG (20%) [Fig. 2a shows example of the negative cases], besides 0/4 cases of Hashimoto's thyroiditis (0%) and 2/7 cases of FA (29%) were positive. All of the positive cases displayed week HBME-1 expression (score +). No statistical significant difference was found among this group as regards HBME-1 expression ($P = 0.08$) but HBME-1 is higher in FA than other benign lesions.

Among the second group, positive HBME-1 expression was observed in 42/50 (84%), which included all the cases of PTC (100%, score +++) [Fig. 2b and c], 10/15 cases of FC (67%, score ++) [Fig. 2d], 6/7 cases of WDT-UMP (86%, score ++) and 4/6 cases of FTs-UMP (67%, score ++) (0%). No statistical significant difference was found among this group as regards HBME-1 expression ($P = 0.06$) but HBME-1 is higher in PTC than for other lesions in the second group. Besides, between the first and the second group, the difference was highly statistically significant ($P = 0.0004$)

3.2.3. 3-Galectin-3 expression in the studied lesions

Gal-3 expression was detected predominantly in the cytoplasm and/or the nucleus.

Among the first group, positive Gal-3 expression was observed in only 4/20 cases (20%), in which 2/5 were cases of MNG (40%, one case score+ and the other is score ++), and 2/7 were cases of FA (29%, score +) [Fig. 3a is an example of the negative FA cases]. All the remaining cases were negative. No statistical difference was found among this group as regards Gal-3 expression ($P = 0.01$).

Among the second group; positive Gal-3 expression was observed in 43/50 cases (86%), which included all the cases of classic PTC (100%, one case score+ one case score+ and 12 cases score+++), all the cases of FVPC (100%, 2 cases score++ and 6 cases score+++) [Fig. 3b], 12/15 cases of FC (80%, one cases score+ 2 cases score++ and 9 cases score+++) [Fig. 3c], 5/7 cases of WDT-UMP (71%, one cases score+ one case score++ and 3 cases score+++) and 4/6 cases of FTs-UMP (67%, 2 cases score++ and 2 cases score+++) [Fig. 3d]. No statistical difference was found among this group as regards Gal-3 expression ($P = 0.02$) but Gal-3 is higher in PTC than other malignant lesions. Besides the relation between the first and the second group was highly statistically significant ($P = 0.005$).

3.2.4. 4-CK19 expression in the studied lesions

CK19 expression was detected in the cell membrane with or without the cytoplasm.

Among the first group, positive expression was observed in only 7/20 cases (35%), which consisted of 1/4 cases of Grave's disease (25%, score +), 2/5 cases of MNG

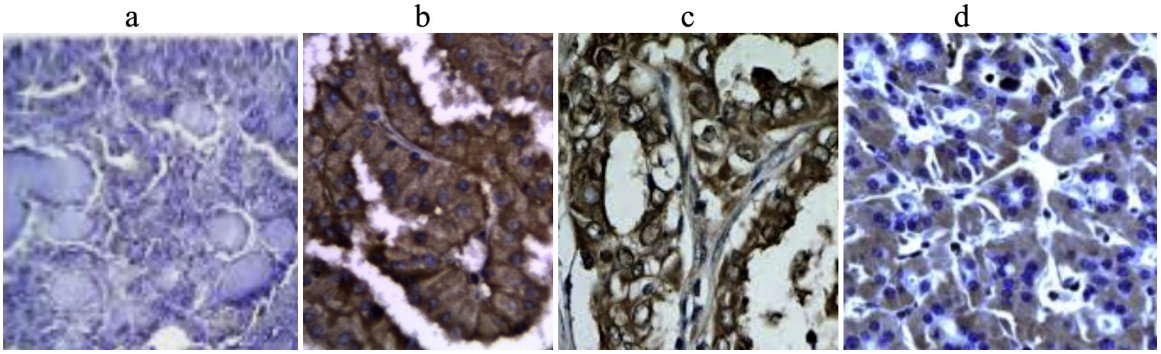

Fig. 2. Immunohistochemical staining of HBME-1 in MNG (−) (a x100), PTC (+++) (b), FVPC (++) (c) and FC (++) (d) [streptavidin biotin 200×].

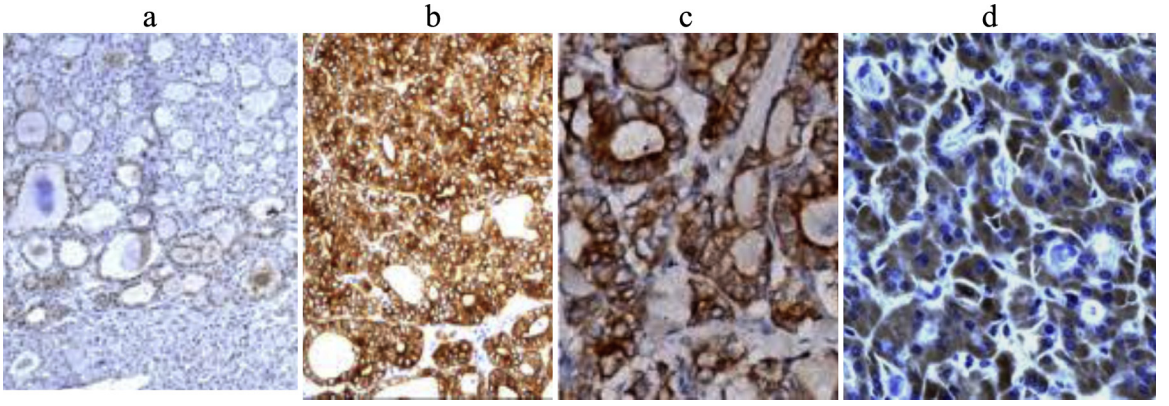

Fig. 3. Immunohistochemical staining of galectin-3 in FA(−) (a x100), FVPC (+++) (b x100), FC (+++) (c) and FT-UMP (+++) (d) [streptavidin biotin 200×].

(40%, one case score+ and the other is score++) [Fig. 4a], and 4/7 cases of FA (57%, one is score+ three are score++). All the remaining cases were negative. No statistical significant difference was found among the first group as regards CK19 expression ($P = 0.4$)

Among the second group, positive CK19 expression was observed in 39/50 (87%), which included all the cases of classic PTC (100%, one case score++, and 13 cases score+++) [Fig. 4b], all the cases of FVPC (100%, 5 cases score++ and 3 cases score+++), 8/15 cases of FC (53%, 2 cases score+ [Fig. 4c], 2 cases score++ and 4 cases score+++), 5/7 cases of WDT-UMP (71%, 2 cases score+ one case score++ and 2 cases score+++) [Fig. 4d] and 4/6 cases of FTs-UMP (67%, one cases score+ one case score++ and 2 cases score+++). It was statistically different among the second group as regards CK19 expression ($P = 0.05$). CK19 is always positive in PTC in contrast to the other malignant lesions. The relation between the first and the second group was highly statistically significant ($P = 0.003$).

3.3. *Specificity and sensitivity of each marker (Table 3)*

Diagnostic validity of CD56 was of highest sensitivity in differentiating FVPC from FA and in differentiating FC from FA (86% in both), while the highest specificity was in differentiating FC from FT-UMP (100%) and in differentiating PTC from benign non-neoplastic lesions (95%).

For HBME-1, diagnostic sensitivity was the greatest in differentiating FVPC from FA (100%), FVPC from FC (100%), PTC from WDT-UMP (100%) and PTC from other benign non-neoplastic lesions (100%), while the specificity was the greatest in differentiating benign from malignant lesions (84%) and PTC from other benign non-neoplastic lesions (85%).

For Gal-3, the highest sensitivity was observed during differentiating FVPC from FA (100%), FVPC from FC (100%), PTC from WDT-UMP (100%) and PTC from benign non-neoplastic lesions (100%), while the specificity was the highest in differentiating benign from malignant lesions (80%) and PTC from benign non-neoplastic lesions (85%).

In CK19, the sensitivity was maximum in differentiating FVPC from FA (100%), FVPC from FC (100%), PTC from WDT-UMP (100%) and PTC from benign non-neoplastic lesions (100%), while the specificity was the highest in differentiating benign from malignant lesions (80%) and PTC from benign non-neoplastic lesions (85%).

3.4. *Combined expression of markers and their diagnostic value (Table 4)*

To improve the diagnostic accuracy of each marker, we calculate the highest sensitivity and specificity for the lesions to analyze the combined effect of the markers.

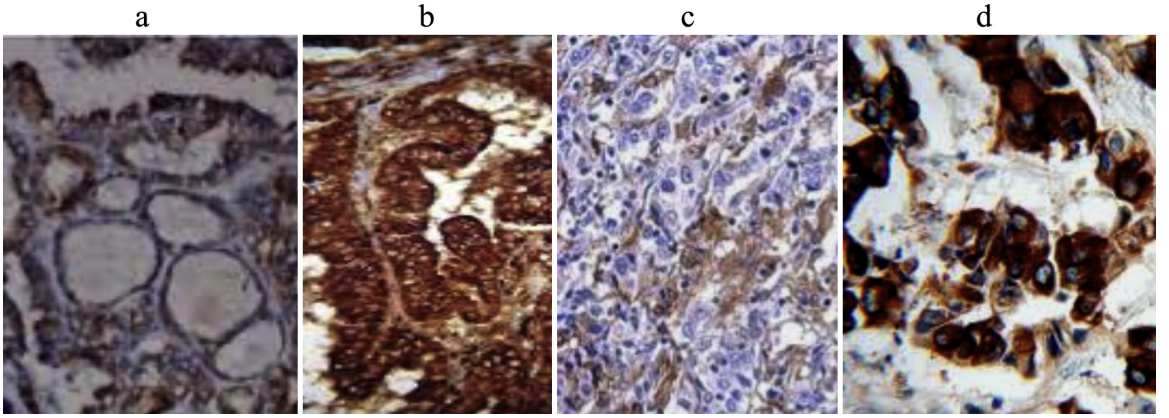

Fig. 4. Immunohistochemical staining of CK19 in MNG (++) (a), PTC (+++) (b), FC (+) (c) and WDT-UMP (+++) (d 400×) [streptavidin biotin 200×].

Table 2
Immunohistochemical expression of the studied markers in different thyroid lesions.

Studied lesions	CD56				*P* value	HBME-1				*P* value	Gal-3				*P* value	CK19				*P* value
	–	+	++	+++		–	+	++	+++		–	+	++	+++		–	+	++	+++	
Normal thyroid tissue (10)	0	0	0	10		10	0	0	0		10	0	0	0		0	0	0	10	
First group (20)																				
Grave's disease (4)	1	0	0	3		3	1	0	0		4	0	0	0		3	1	0	0	
Multinodular goiter (5)	1	0	0	4		4	1	0	0		3	1	1	0		3	1	1	0	
Hashimoto's thyroiditis (4)	1	0	0	3		4	0	0	0		4	0	0	0		4	0	0	0	
Thyroid adenoma (7)	1	0	0	6		5	2	0	0		5	2	0	0		3	1	3	0	
P value among the first group			**0.9**		**0.001**			**0.08**		**0.0004**			**0.01**		**0.005**			**0.4**		**0.003**
Second group(50)																				
Papillary carcinoma																				
Classic (14)	12	0	2	0		0	0	0	14		0	1	1	12		0	0	1	13	
FVPC (8)	7	1	0	0		0	0	0	8		0	0	2	6		0	0	5	3	
Follicular carcinoma (15)	13	2	0	0		5	0	10	0		3	1	2	9		7	2	2	4	
WDT-UMP (7)	7	0	0	0		1	0	6	0		2	1	1	3		2	2	1	2	
FT-UMP (6)	6	0	0	0		2	0	4	0		2	0	2	2		2	1	1	2	
P value among the second group			**0.5**					**0.06**					**0.02**					**0.05**		

Table 3
Sensitivity and specificity of each marker in differential diagnosis of thyroid lesions.

Studied marker	Benign vs. malignant		FVPC vs. FA		FC vs. FA		FVPC vs. FC		FC vs. FT-UMP		PTC vs. WDT-UMP		PTC vs. benign non-neoplastic lesions	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
CD56	80	90	86	88	86	87	13	87	13	100	14	14	77	95
HBME-1	80	84	100	71	67	71	100	33	67	33	100	14	100	85
Gal-3	80	80	100	71	80	71	100	20	80	33	100	29	100	85
CK19	65	78	100	43	53	43	100	47	53	33	100	29	100	77

Table 4
Sensitivity and specificity of combined markers in differential diagnosis of thyroid lesions.

Studied marker	Benign vs. malignant		FVPC vs. FA		FC vs. FA		FVPC vs. FC		FC vs. FT-UMP		PTC vs. WDT-UMP		PTC vs. benign non-neoplastic lesions	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
CD56 and HMBE!	95	98	95	90	90	85	66	56	57	60	77	65	100	90
HBME-1 and Galectin-3	85	87	100	100	85	80	100	40	80	45	100	40	100	100
CD56 and Galectin-3	80	90	95	80	90	94	55	90	95	100	80	50	95	96
CD56 or CK19	70	82	100	55	80	60	100	90	50	70	75	30	100	85
CK19 or Galectin-3	74	81	95	64	75	65	100	77	74	55	100	57	95	88

To differentiate benign from malignant, we analyze the specificity and sensitivity for CD56 or HBME-1, the sensitivity improved to 95% and the specificity improved to 98%. For FVPC vs. FA, we calculate the specificity and sensitivity for HBME-1 or Galectin-3 or CD56, the sensitivity was 100% and the specificity improved to 100%. To differentiate FC from FA, we calculate the specificity and sensitivity for CD56 or Galectin-3, the sensitivity was 90% and the specificity improved to 94%, while to differentiate FVPC from FC, we calculate the specificity and sensitivity for CD56 or CK19, the sensitivity was 100% and the specificity improved to 90%. To diagnose FC from FT-UMP, we analyze the specificity and sensitivity for CD56 or Galectin-3, the sensitivity improved to 95% and the specificity improved to 100%. On the other hand, to differentiate PTC from WDT-UMP, we examine the specificity and sensitivity for CK19 or Galectin-3, the sensitivity was 100% and the specificity improved to 57%. Lastly, to differentiate PTC from benign non-neoplastic lesions, we check the specificity and sensitivity for HBME-1 or Galectin-3, the sensitivity was 100% and the specificity improved to 100%.

4. Discussion

A somewhat common dilemma is encountered with tumors showing follicular growth pattern. Presence or absence of capsular and/or vascular invasion distinguishes benign from malignant follicular tumors, but identification of this finding can be challenging due to incomplete capsular penetration. Another situation is encountered when some of the nuclear features of PTC are present. Also in the absence of papillary architecture, distinguishing the FVPCs from cellular adenomatous nodules may be challenging [21].

For all of the aforementioned reasons, investigators have focused during the last several years on finding IHC markers that can help in the distinction for these challenging cases [5,23].

CD56 has been reported to be related to the differentiation of the follicular epithelium and many previous studies reported high CD56 expression in normal thyroid tissue and benign thyroid follicular lesions [10,24]. In accordance with those studies, we currently report a high positive CD56 expression in normal thyroid tissue and the benign group. On the other hand, negative CD56 expression was observed in 90% of the second group cases. Similarly, previous studies reported negative CD56 expression in all or most of their studied PTC cases [5,24].

Based on the previous results and in the light of our finding, there was no statistically significant difference between CD56 expression among each group but CD56 distinguished the second group from the first group so it can be used to differentiate FC from FA, FVPCs from other benign nodules and PTC from benign lesions showing papillary structures. Therefore we were able to emphasize that lack of CD56 expression in FVPCs and PTC was very helpful in their discrimination from other follicular lesions. These data were in accordance with Arturs et al. [5].

The sensitivity and the specificity of CD56 as a negative marker were very impressive in distinguishing benign lesions from malignant lesions, FVPCs from FA, also in differentiating FC from FA and in distinguishing PTC from other benign non-neoplastic lesions. On the other hand, the highest specificity was in differentiating FC from FT-UMP. These results were in agreement with Dina et al. [7]. On the contrary, Etem et al. [25] found no statistical difference between FVPCs and other follicular tumors (FTs-UMP, FA and FC) as regards CD56 expression.

HBME-1 has been reported to be one of the most promising markers [14]. Among the benign group, positive HBME-1 expression was weakly observed in 20%, 29% of FA were positive and it is higher in FA than other benign lesions. This was differing from Arturs et al. [5] who found no HBME-1 in benign lesions.Among the malignant group, positive expression was observed in 84%, including all cases of PTC and 67% of FC. Miettinen et al. [26] showed that all FC were positive for HBME-1, although this phenomenon could be observed in only 28% of FA. In Yasuhiro et al. [4] and Nasr et al. [21] study, there was a significant difference in the incidence of HBME-1 positivity between FC and FA.

In the present study, HBME-1 expression is higher in PTC than other lesions in the second group, so it can be used to differentiate FVPC from FC. Besides the difference between the first and the second group was highly statistically significant ($P = 0.0004$), so we can use it to differentiate PTC from other benign lesions and FA from FVPTC. These results were in agreement with Young et al. [12].

HBME-1 seems to be a sensitive marker for thyroid carcinoma, especially PTC. This was in accordance with Prasad et al. [14] and Nasr et al. [21]. The sensitivity and the specificity for HBME-1 in distinguishing malignant from benign were 80% and 84% respectively and this was in agreement with Husain et al. [11] who showed that the sensitivity and the specificity of HBME-1 to distinguish benign from malignant lesions was one of the highest among all markers. Cheung et al. [27] reported HBME1 positivity in 70% classic PTC and 45% FVPC with no expression in nodular hyperplasia cases and FA. Similarly, Prasad et al. [14] demonstrated HBME1 expression in 85% PTC.

In distinguishing FVPC from FA, the sensitivity and the specificity were 100% and 71%, respectively while in differentiating PTC from other benign non-neoplastic lesions, they were 100% and 85% respectively. On the other hand, HBME-1 showed highest sensitivity (100%) in distinguishing FVPC from FC and PTC from WDT-UMP, but the specificity was low. Husain et al. [22] study concluded that HBME-1 is not a very good marker to distinguish adenomas from thyroid carcinomas with over half of the adenomas expressing this marker. Also Mauro et al. [6] in a study of WDT-UMP found that a diffuse and strong expression of HBME-1, is observed. So, we and others [1,6,22] can say that although HBME-1 contributes to the diagnosis of both FC and WDT-UMP, it cannot be applied alone in differential diagnosis of follicular-patterned lesions due to its low specificity.

In the present study, Gal-3 positive rate in two groups was 20% and 86%, respectively. Gasbarri et al. [28] observed that galectin-3 is never expressed in benign thyroid lesions. Saggiorato et al. [29] observed only 4/52 FA expressing Gal-3 immunopositivity, whereas all thyroid cancers that those investigators analyzed were immunopositive for Gal-3. In

the same way, Orlandi et al. [30] reported that although all the thyroid cancers that they analyzed were Gal-3 immunopositive, only 3/29 FA exhibited such positivity. Some authors consider true Gal-3-positive FA as an indication of potentially early or incipient carcinoma, in which the capsular and/or vascular invasion cannot be histologically observed as yet [31].

On the other hand, some recent studies demonstrated that Gal-3 is highly expressed in benign thyroid lesions and in normal thyroid tissue [12,14]. These discrepancies may be related to the different antibody detection systems. In the thyroid gland, endogenous biotin is invariably expressed in thyrocytes. Thus, a biotin-based detection system may provide false positive results. It has been suggested that Gal-3 immunodetection may be a useful adjunct in the distinction between benign and malignant thyroid tumors, only if performed in a biotin-free detection system [12].

In the second group, all cases of PTC were positive, and Gal-3 is higher in PTC than other malignant lesions, so we can use it to differentiate FVPC from FC; therefore Gal-3 has been consistently a very sensitive marker for PTC [14].In the current study, the relation between the first and the second group was highly significant and this was in agreement with Qingbin et al. [23], so it can be used to differentiate FC from FA, FVPC from FA and PTC from other benign lesions.

In a study by Bartolazzi et al. [32], the sensitivity and the specificity of Gal-3 in thyroid carcinomas were 99% and 98%, respectively. In Husain et al. [11] study the values were 92.6% and 77.3%, respectively. In the present study, we observed high sensitivity and specificity of Gal-3 in differentiating malignant from benign, FVPC from FA, FC from FA, and PTC from other benign non-neoplastic lesions. Previous studies revealed similar data and recommended its use to identify thyroid malignancies including FC and PTC [33,34]. On the other hand, the highest sensitivity for Gal-3 was also observed in distinguishing FVPC from FC and PTC from WDT-UMP (100% both) but the specificity was low for both (20% and 29% respectively).

Positive CK19 expression was observed in 40% of MNG and 57% of FA. Some studies have reported negative CK19 staining in benign thyroid lesions [15], while Cheung et al. [27] demonstrated that 20% of the nodular goiters were focally CK19 positive. In Debdas et al. [9] study, 50% of MNG and 75% of FA were positive but focal. The study by Nasr et al. [21] also noted a 68% CK19 positivity in benign lesions, but staining intensity was weak. Sahoo et al. [35] also found CK19 positivity in 100% of FA. In all these cases, CKI9 staining was patchy and moderate. We did not find any strong positive FA. Nasr et al. [21] also demonstrated the weak CK19 status in 5/6 FAs. Guyetant et al. [36] showed that 90% of the FAs were focally positive for CK19. The significance of focal expression of CK19 in some FA is unknown. Further studies are necessary to show whether these tumors have a different clinical behavior or molecular profile.

On the other hand, among the second group, the positive CK19 expression was observed for all the cases of PTC, 53% of FC, 71% of WDT-UMP and 67% of FTs-UMP. According to Sahoo et al. [35] and Guyetant et al. [36] all cases of PTC showed strong positivity for CK19. The study done by Cheung et al. [27] observed that 57% of FVPC were positive for CK19, while Yoon et al. [3] study showed that CK19 might be positive markers for the FVPC and they are not so useful for classic PC. In the current study, it was observed that CK19 can differentiate FVPC from FC and PTC from both WDT-UMP and FTs-UMP, On the other hand it can differentiate PTC from benign lesions because the difference between the first and the second group was statistically significant.

The sensitivity and the specificity as regards CK19 in distinguishing malignant from benign were 65% and 78% respectively but in distinguishing PTC from other benign non-neoplastic lesions, the sensitivity and the specificity were 100% and 77% respectively. The sensitivity was 100% when it was used to distinguish FVPC from FA, FVPC from FC and PTC from WDT-UMP but the specificity was low. Other studies showed a high sensitivity and specificity of CK19 in PTC [14,26]. They confirmed that CK19 is a useful marker for differentiating PTC from papillary hyperplasia. However, they also identified expression of CK19 in follicular neoplasms and hence, in these studies and in our analyses CK-19 alone was not useful in the diagnosis of follicular thyroid lesions. The chief utility of CK19 lies in its high sensitivity for PTC. Negative staining for CK19, therefore, is strong evidence against PTC.

In summary, as no marker by itself has a superior diagnostic value, a combination of markers may be more accurate than any single marker. We attempted to identify the best combination of markers with the greatest specificity and sensitivity. CD56 with HBME-1 were the best to differentiate benign from malignant, CD56, HBME-1 and Gal-3 were the best in differentiating FVPC from FA, while CD56 and Gal-3 were the best to distinguish FC from FA. CD56 was the most specific in distinguishing FVPC from FC while the sensitivity was the same as regards the other three markers. To distinguish FC from FT-UMP, the best were CD56 and Gal-3 while to differentiate PTC from WDT-UMP, the best were Gal-3 and CK19. Lastly to differentiate PTC from other benign non-neoplastic lesions, the best were HBME-1, Gal-3 and CD56. The sensitivity and the specificity were increased when we used combinations of this panel together. Our recent observations encouraged us to assess the possible value of the (CD56, HBME-1, Gaectin-3 and CK19) panel in the differential diagnosis of the studied thyroid nodules with a better sensitivity and specificity. This panel was able to discriminate benign from malignant lesions, PTC, FVPCs, FA, FC, FT-UMP and WDT-UMP among other similar follicular cell-derived thyroid lesions.

Conflict of interest

The author declares that she has no conflict of interest.

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